

Document made available under the Patent Cooperation Treaty (PCT)

International application number: PCT/US05/002262

International filing date: 24 January 2005 (24.01.2005)

Document type: Certified copy of priority document

Document details: Country/Office: US
Number: 60/538,698
Filing date: 23 January 2004 (23.01.2004)

Date of receipt at the International Bureau: 03 March 2005 (03.03.2005)

Remark: Priority document submitted or transmitted to the International Bureau in compliance with Rule 17.1(a) or (b)



World Intellectual Property Organization (WIPO) - Geneva, Switzerland
Organisation Mondiale de la Propriété Intellectuelle (OMPI) - Genève, Suisse

1287307

THE UNITED STATES OF AMERICA

TO ALL TO WHOM THESE PRESENTS SHALL COME:

UNITED STATES DEPARTMENT OF COMMERCE

United States Patent and Trademark Office

February 18, 2005

THIS IS TO CERTIFY THAT ANNEXED HERETO IS A TRUE COPY FROM THE RECORDS OF THE UNITED STATES PATENT AND TRADEMARK OFFICE OF THOSE PAPERS OF THE BELOW IDENTIFIED PATENT APPLICATION THAT MET THE REQUIREMENTS TO BE GRANTED A FILING DATE.

APPLICATION NUMBER: 60/538,698

FILING DATE: *January 23, 2004*

RELATED PCT APPLICATION NUMBER: PCT/US05/02262



Certified by

Don W. Dudas

Under Secretary of Commerce
for Intellectual Property
and Director of the United States
Patent and Trademark Office

04772 U.S. PTO
012304

PTO/SB/16 (08-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE
Under the Paperwork Reduction Act of 1995, no persons are required to respond to a collection of information unless it displays a valid OMB control number.

PROVISIONAL APPLICATION FOR PATENT COVER SHEET

This is a request for filing a PROVISIONAL APPLICATION FOR PATENT under 37 CFR 1.53(c).

Express Mail Label No. **EL084752585US**

| INVENTOR(S) | | | | | |
|---|--|--|--|---|--|
| Given Name (first and middle (if any)) | | Family Name or Surname | | Residence (City and either State or Foreign Country) | |
| Yulin | | Ren | | Athens, Ohio | |
| Additional inventors are being named on the _____ separately numbered sheets attached hereto | | | | | |
| TITLE OF THE INVENTION (500 characters max) | | | | | |
| PGG SEPARATION AND PURIFICATION | | | | | |
| Direct all correspondence to: CORRESPONDENCE ADDRESS | | | | | |
| <input checked="" type="checkbox"/> Customer Number: 24024 | | | | | |
| OR | | | | | |
| <input type="checkbox"/> Firm or Individual Name | | | | | |
| Address | | | | | |
| Address | | | | | |
| City | | State | | Zip | |
| Country | | Telephone | | Fax | |
| ENCLOSED APPLICATION PARTS (check all that apply) | | | | | |
| <input checked="" type="checkbox"/> Specification Number of Pages 9 | | <input type="checkbox"/> CD(s), Number _____ | | | |
| <input checked="" type="checkbox"/> Drawing(s) Number of Sheets 1 | | <input type="checkbox"/> Other (specify) _____ | | | |
| <input type="checkbox"/> Application Date Sheet. See 37 CFR 1.76 | | | | | |
| METHOD OF PAYMENT OF FILING FEES FOR THIS PROVISIONAL APPLICATION FOR PATENT | | | | | |
| <input checked="" type="checkbox"/> Applicant claims small entity status. See 37 CFR 1.27. | | | | FILING FEE Amount (\$) | |
| <input type="checkbox"/> A check or money order is enclosed to cover the filing fees. | | | | 80.00 | |
| <input checked="" type="checkbox"/> The Director is hereby authorized to charge filing fees or credit any overpayment to Deposit Account Number: 03-0172 | | | | | |
| <input type="checkbox"/> Payment by credit card. Form PTO-2038 is attached. | | | | | |
| The invention was made by an agency of the United States Government or under a contract with an agency of the United States Government. | | | | | |
| <input type="checkbox"/> No. | | | | | |
| <input type="checkbox"/> Yes, the name of the U.S. Government agency and the Government contract number are: _____ | | | | | |

[Page 1 of 2]

Respectfully submitted,

SIGNATURE

TYPED or PRINTED NAME **Kristin J. Frost**

TELEPHONE **216-622-8895**

Date **January 23, 2004**

REGISTRATION NO. **50,627**

(if appropriate)

Docket Number: **27211/04094**

USE ONLY FOR FILING A PROVISIONAL APPLICATION FOR PATENT

This collection of information is required by 37 CFR 1.51. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 8 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS ADDRESS. SEND TO: Mail Stop Provisional Application, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

If you need assistance in completing the form, call 1-800-PTO-9199 and select option 2.

Express Mail Label No. **EL084752585US**

| INVENTOR(S) | | |
|--|------------------------|---|
| Given Name (first and middle [if any]) | Family Name or Surname | Residence (City and either State or Foreign Country) |
| Yulin | Ren | Athens, Ohio |
| Additional inventors are being named on the _____ separately numbered sheets attached hereto | | |
| TITLE OF THE INVENTION (500 characters max) | | |
| PGG SEPARATION AND PURIFICATION | | |

| CERTIFICATE OF EXPRESS MAILING | |
|---|--|
| Date of Deposit: <u>January 23, 2004</u> | |
| I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" dldldldld under 37 CFR 1.10 on the date indicated above and is addressed to Mail Stop Prov. Patent App., Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450. | |
| Robert Watts | |
| Name of Person Signing (Type or Print) | |
| Signed: <u>Robert Watts</u> | |

"EXPRESS MAIL" Mailing Label No.: EL084752585US

Date of Deposit: January 23, 2004

I hereby certify that this paper or fee is being deposited with the United States Postal Service "Express Mail Post Office to Addressee" service under 37 CFR 1.10 on the date indicated above and is addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

Robert Watts

Name of Person Signing (Type or Print)

Signed: Robert Watts

27211/04094

PGG SEPARATION AND PURIFICATION

BACKGROUND OF THE INVENTION

[0001] PGG and its analogues show anti-diabetic and other beneficial bioactivities that make them useful in the development of new drugs. Typically, for FDA approval of new drugs, the purity of the drugs must be higher than 95%. Currently, there are no efficient, cost-effective methods of preparing and purifying PGG or its analogues on a gram to kilogram scale. Until now, chromatographic purification methods have been the only known methods for producing PGG or its analogues at a purity of 95% or greater. Chromatographic methods, however, are both expensive and not amenable to large scale purification of PGG and its analogues. Production of PGG on an industrial scale using currently available methods is prohibitively expensive.

[0002] Accordingly, there exists a need for methods of separation and purification of PGG and its analogues that are less expensive than currently known methods, and amenable to larger scale production.

SUMMARY OF THE INVENTION

[0003] The present invention provides a simple, inexpensive, and efficient method for separation and purification of the α - and β - forms of penta-O-galloyl-D-glucose (PGG). Specifically the present invention provides methods for separating α -PGG or β -PGG from a mixture that contains

PGG and other chemicals. The methods of the present invention, unlike previous separation and purification methods, require no HPLC step. Because no HPLC step is required, the methods of the present invention are amenable to producing large quantities of purified α -PGG and β -PGG.

[0004] In one embodiment, the present invention provides a method of separating α -PGG from a mixture containing α -PGG and β -PGG. In another aspect, the present invention provides a method for separating β -PGG from a mixture containing both α -PGG and β -PGG. The methods of the present are especially suitable for separating α -PGG from a mixture of α -PGG and β -PGG that contains more than 50% α -PGG, or separating β -PGG from a mixture of α -PGG and β -PGG that contains more than 50% β -PGG.

[0005] The present invention further provides methods of purifying α -PGG and β -PGG to purities of greater than 95%. In one embodiment, the method provides α -PGG or β -PGG at greater than 98% purity. The present invention also provides methods of growing single crystals of α -PGG and single crystals of β -PGG.

[0006] The present invention also provides methods for the separation and purification of analogues of α - and β -PGG in which the glucose part of the PGG is substituted by other sugars, preferably hexoses, pentoses, or tetroses. Preferred hexoses include galactose, mannose, idose, talose, altrose, allose, gulose, fructose, or similar. Preferred pentoses include xylose, ribose, arabinose, and lyxose. Preferred tetroses include threose and erythrose. The methods of the present invention are able to separate the α - and β -PGG analogues from mixtures of α and β , preferably, separating the α -form of the analogue from mixtures that contain more than 50% of the α -form and separating the β -form of the analogue from mixtures that contain than 50% β -form. In both cases, the α -form and the β -form can be purified to a level of 95% or greater purity. The present invention further provides methods for producing the α -form or the β -form at purities of 98% or greater.

[0007] The present invention further provides methods for the separation and purification of analogues of α - and β -PGG in which the glucose part of the PGG is substituted by sugar analogues, of glucose, other hexoses, pentoses, or tetroses, in which the ring oxygen of the sugar analogue is substituted by carbon, nitrogen, or sulfur. With respect to these analogues, the

methods of the present invention are able to separate α -PGG analogues and β -PGG analogues, preferably when there is more than 50% of the α -PGG analogue or more than 50% of the β -PGG analogue present, respectively, in the impure starting material. In accordance with the methods of the present invention, both the α -PGG analogues and the β -PGG analogues can be purified to purities of 95% or greater. The present invention further provides methods for producing the α -form or the β -form at purities of 98% or greater.

[0008] The present invention also provides methods of separation and purification of analogues of α - and β -PGG wherein the gallic acid part of the PGG is replaced by other phenols. Preferred phenols include, but are not limited to 2,3-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and 3,5-dihydroxybenzoic acid. In accordance with the methods of the present invention, these α -PGG analogues and β -PGG analogues may be separated from mixtures of α -PGG analogues and β -PGG analogues. Preferably, the starting material for the purification of the α -form of the analogues contains more than 50% of the α -form, and the starting material for the purified β -form of the analogue contains more than 50% of the β -form. Additionally, both the α -PGG analogues and β -PGG analogues having the gallic acid replaced by another phenol may be purified to 95% or greater purity, or 98% or greater purity.

[0009] The method for separation of α -PGG from a mixture of α -PGG and β -PGG comprises the steps of (a) adding water to a sample containing 50% or more α -PGG and 50% or less β -PGG; (b) mixing the PGG and water to dissolve the PGG; (c) filtering out any undissolved particles; and (d) allowing the filtered solution to stand undisturbed until crystals form. Preferably the water used is double distilled water. A preferable ratio of water to PGG is about 20 mL of water for about 1 g of PGG. Preferably, the mixing in step (b) is done for about 5 minutes. Optionally, the mixing step (b) may be done at an elevated temperature, to aid in dissolution of the PGG. Preferably, step (b) is carried out at 80°C, by placing the flask in a water bath incubator, or similar. Preferably, the filtration is done through a 45 μ filter. Preferably, the flask contained the filtered solution is allowed to stand at room temperature, however, the flask may be kept at a lower temperature to speed up formation of the crystals. The method may be repeated to obtain purer α -PGG. In accordance with the present invention, this method for purification of α -PGG may also be used for analogues of α -PGG, including, but not limited to analogues in which the glucose of the PGG is substituted by a hexose, pentose, or tetrose; analogues in which the

glucose is substituted by sugar analogues in which the ring oxygen is substituted by carbon, nitrogen, or sulfur; and analogues in which the gallic acid portion of the PGG is substituted by other phenols.

[0010] The method for separation of β -PGG from a mixture of α -PGG and β -PGG comprises the steps of (a) adding acetone to a sample containing 50% or more β -PGG and 50% or less α -PGG; (b) mixing the PGG and acetone to dissolve the PGG; (c) filtering out any undissolved particles; and (d) allowing the filtered solution to stand undisturbed until crystals form. Preferably, the acetone is added to the PGG at a ratio of about 5 mL water for about 1 g PGG. Preferably, the mixing in step (b) is done for about 5 minutes. Optionally, the mixing step (b) may be done at an elevated temperature, to aid in dissolution of the PGG. Preferably, step (b) is carried out at 80°C, by placing the flask in a water bath incubator, or similar. Preferably, the filtration is done through filter paper. Preferably, the flask contained the filtered solution is allowed to stand at room temperature, however, the flask may be kept at a lower temperature to speed up formation of the crystals. In accordance with the present invention, this method for purification of β -PGG may also be used for analogues of β -PGG, including, but not limited to analogues in which the glucose of the PGG is substituted by a hexose, pentose, or tetrose; analogues in which the glucose is substituted by sugar analogues in which the ring oxygen is substituted by carbon, nitrogen, or sulfur; and analogues in which the gallic acid portion of the PGG is substituted by other phenols.

[0011] The method for preparing single crystal α -PGG comprises the steps of (a) adding water to a sample of pure (95% or greater) α -PGG; (b) mixing the α -PGG and water to dissolve the α -PGG; (c) filtering out any undissolved particles, placing the filtered solution in a clean vessel; and (d) maintaining the filtered solution undisturbed until crystals appear. Preferably, double distilled water is used in the method of preparing a single crystal of α -PGG. Preferably, the water is added to the α -PGG at a ratio of about 100 mL of water for about 1.0 g α -PGG. Preferably, the mixing in step (b) is done for about 5 minutes. Optionally, the solution may be heated during mixing step (b) to aid in dissolution of the α -PGG. Preferably, mixing step (b) is carried out at 80°C. Preferably, solution is filtered through filter paper in step (c). Preferably, step (d) is carried out at room temperature, for about 15 days. In accordance with the present invention, this method for preparing single crystals of α -PGG may also be used for analogues of

α -PGG, including, but not limited to analogues in which the glucose of the PGG is substituted by a hexose, pentose, or tetrose; analogues in which the glucose is substituted by sugar analogues in which the ring oxygen is substituted by carbon, nitrogen, or sulfur; and analogues in which the gallic acid portion of the PGG is substituted by other phenols.

[0012] The method for preparing single crystal β -PGG comprises the steps of (a) adding acetone to a sample of pure (95% or greater) β -PGG; (b) mixing the β -PGG and acetone to dissolve the β -PGG; (c) filtering out any undissolved particles, placing the filtered solution in a clean vessel; and (d) maintaining the filtered solution undisturbed until crystals appear. Preferably, the ratio of acetone to PGG is about 50 mL of acetone per about 1.0 g β -PGG. Preferably, the mixing in step (b) is done for about 5 minutes. Optionally, the solution may be heated during mixing step (b) to aid in dissolution of the PGG. Preferably, mixing step (b) is carried out at 80°C. Preferably, solution is filtered through filter paper in step (c). Preferably, step (d) is carried out at room temperature for about 20 days. In accordance with the present invention, this method for preparing single crystals of β -PGG may also be used for analogues of β -PGG, including, but not limited to analogues in which the glucose of the PGG is substituted by a hexose, pentose, or tetrose; analogues in which the glucose is substituted by sugar analogues in which the ring oxygen is substituted by carbon, nitrogen, or sulfur; and analogues in which the gallic acid portion of the PGG is substituted by other phenols.

BRIEF DESCRIPTION OF THE FIGURES

[0013] **Figure 1** shows the crystal structures of α -PGG (left) and β -PGG (right) under differential interference contrast microscope.

DETAILED DESCRIPTION OF THE INVENTION

[0014] The present invention provides methods for separation and purification of the α - and β -isomers of PGG by crystallization. The methods of the present invention may also be used to separate the α - and β -isomers of many different PGG analogues, include some that are potentially useful as pharmaceutical agents. The methods of the present invention may be used for quantities ranging from laboratory scale to kilogram quantities, up to ton quantities, while still producing isomers with purities of 95% or greater. Additionally, the methods of the present

invention are very cost effective and environmentally friendly since water is the only solvent needed. The methods of the present invention may also be used to produce single crystals of α -PGG, β -PGG or analogues thereof.

[0015] Since the methods of the present invention achieve separation and purification of α - and β -PGG and analogues thereof on a kilogram to ton scale, the methods are suitable for industrial application. Furthermore, the methods of the present invention are inexpensive—the only solvent needed is water, and standard instrumentation can be used with the inventive methods. In addition, the process is performed at room temperature, which makes expensive and time-consuming heating and/or cooling steps unnecessary. The method is also environmentally friendly since no organic solvents are necessary, and the process can be run without heating and cooling.

[0016] Until the present invention, the only method available to produce high purity α -PGG and β -PGG isomers was high performance liquid chromatography (HPLC). HPLC has many disadvantages, making it unsuitable for separation of large quantities of material. HPLC can only be used for the separation of milligram to gram-size quantities of PGG. In addition, it is slower and far more expensive than crystallization, requiring a complicated HPLC system that costs at least \$25,000 to \$30,000 to purchase. Furthermore, large quantities of solvent must be used to run the HPLC and significant amounts of compound must be sacrificed (discarded) to maintain high purity, resulting in a low yield of recovered material.

[0017] Using the methods of the present invention, PGG and its analogues may be separated and purified in water or water-based solvent systems, efficiently with very low cost and high yield. The methods of the present invention reduce the total cost to manufacture isomers of PGG and its analogues, at a purity of at least 95%, by more than 95%.

[0018] The methods of the present invention are useful for separating α -PGG from a mixture containing α -PGG and β -PGG and for separating β -PGG from a mixture containing both α -PGG and β -PGG. The methods of the present are especially suitable for separating α -PGG from a mixture of α -PGG and β -PGG that contains more than 50% α -PGG, as well as separating β -PGG from a mixture of α -PGG and β -PGG that contains more than 50% β -PGG.

[0019] The crystallization methods of the present invention provide α -PGG and β -PGG, and analogues thereof with purities of greater than 95%. In another embodiment, the methods of the present invention produce α -PGG or β -PGG, or analogues thereof, with purities of 98% or greater. The present invention also provides methods of growing single crystals of α -PGG and single crystals of β -PGG.

[0020] The present invention also provides methods for the separation and purification of analogues of many analogues of α - and β -PGG. In one such analogue, the glucose part of the PGG is substituted by other sugars, preferably hexoses, pentoses, or tetroses. Preferred hexoses include galactose, mannose, idose, talose, altrose, allose, gulose, fructose, or similar. Preferred pentoses include xylose, ribose, arabinose, and lyxose. Preferred tetroses include threose and erythrose. The methods of the present invention are able to separate the α - and β -PGG analogues from mixtures of α and β , including the case where there is more than 50% of the α -form present and the case when there is more than 50% β -form present. In both cases, the α -form and the β -form can be purified to a level of 95% or greater purity.

[0021] A second class of PGG-analogues suitable for the separation and purification methods of the present invention are analogues of α - and β -PGG in which the glucose part of the PGG is substituted by sugar analogues, of glucose, other hexoses, pentoses, or tetroses, in which the ring oxygen of the sugar analogue is substituted by carbon, nitrogen, or sulfur. With respect to these analogues, the methods of the present invention are able to separate α -PGG analogues and β -PGG analogues. The methods are suitable for mixtures where there is more than 50% of the α -PGG analogue or more than 50% of the β -PGG analogue. In accordance with the methods of the present invention, both the α -PGG analogues and the β -PGG analogues can be purified to purities of 95% or greater.

[0022] The present invention also provides methods of separation and purification of analogues of α - and β -PGG wherein the gallic acid part of the PGG is replaced by other phenols. Preferred phenols include, but are not limited to 2,3-dihydroxybenzoic acid, 3,4-dihydroxybenzoic acid, and 3,5-dihydroxybenzoic acid. In accordance with the methods of the present invention, these α -PGG analogues and β -PGG analogues may be separated from mixtures of α -PGG analogues and β -PGG analogues containing either more than 50% of the α -form or mixtures containing

more than 50% of the β -form. Additionally, both the α -PGG analogues and β -PGG analogues having the gallic acid replaced by another phenol may be purified to 95% or greater purity.

[0023] METHODS

[0024] Standard Operation Procedure of Crystallization of α -PGG or its analogues

[0025] Crystallization is carried out on a laboratory scale as follows: (1) 1.0 g of a sample containing α -PGG having a purity of 50% or more is added to a 100-mL flask. (2) 20 mL of double distilled water is then added to the flask. (3) The flask is placed in an 80°C water bath incubator for about 5 minutes, and gently shaken to dissolve the sample. (4) Any undissolved particles are removed using a 0.45 μ membrane filter and a 60 mL plastic syringe through a 18G1½ needle. The filtered solution is added to a clean flask. (5) The flask is kept undisturbed at room temperature for approximately 5 – 7 days, until some white crystals appear.

[0026] The speed of crystallization is affected by temperature, and crystallization may be accelerated by keeping the flask at a temperature below room temperature.

[0027] If higher purity is desired, filter the crystals and repeat steps 1 – 5. A sample with purity higher than 98% may be obtained by repeating these steps more than four times.

[0028] To scale up the procedure, add 20 mL of double distilled water for every 1 g of sample and follow the procedure as outlined above.

[0029] Standard Operation Procedure for Growing Single Crystals of α -PGG

[0030] (1) Add 1.0 g of 95% or greater purity α -PGG to a 200 mL flask. (2) Add 100 mL double distilled water to the flask. (3) Put the flask in an 80°C water bath incubator for about 5 minutes, gently shake the flask to dissolve the sample. (4) Remove any undissolved particles with filter paper, adding the clear, filtered solution to a clean flask. (5) Leave the flask undisturbed, at room temperature, for about 15 days, until some thin, colorless, needle crystals appear. (6) Filter the crystals and store them in a sealed flask.

[0031] Standard Operation Procedure for Crystallization of β -PGG

[0032] The procedure for crystallization of β -PGG is as follows: (1) Add 1.0 g of sample contain β -PGG, having 50% or greater purity, into a 10 mL flask. (2) Add 5.0-mL of acetone to the flask. (3) Place the flask in an 80°C water bath incubator for approximately 10 minutes, gently shake the flask to dissolve the sample. (4) Filter the solution through filter paper, adding the filtered solution to a clean flask. (5) Leave the flask undisturbed at room temperature for about 15 days, until some colorless needle crystals appear. (6) Filter the crystals and repeat steps 1 – 5 if higher purity is desired. For scaling up the procedure, add acetone in the proportion of 1 g sample to 5 g acetone and crystallize the β -PGG.

[0033] Standard Operation Procedure for Growing Single Crystals of β -PGG

[0034] (1) Add 1.0 g sample containing pure (95% or greater) β -PGG to a 100 mL flask. (2) Add 50 mL of acetone to the flask. (3) Place the flask in an 80°C water bath incubator for about 10 minutes, gently shaking the flask to dissolve the sample. (4) Filter the solution through filter paper, adding the filtered solution to a clear flask. (5) Leave the flask undisturbed at room temperature for about 20 days, until some colorless needle crystals appear. (6) Filter the crystals and store the crystals in a sealed flask.



α -PGG crystals (x 50)

Long, thin and needle-like



β -PGG crystals (x 50)

Short and rod-like